

Figure 5 shows EAST inhibition curves of the Phl p 5b mutants using the allergic patient serum pool II/17.

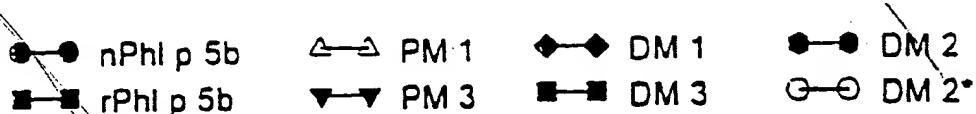


Figure 6 shows release of histamine from human basophils after reaction with the allergens and allergen mutants.

Please delete pages 37-41 in their entirety.

On page 48, the first full paragraph has been deleted in its entirety.

On page 49, the first full paragraph has been amended as follows:

TABLE 7:

E3

Proliferative reaction of Phl p 5b-reactive T cell clones (TCCs) from allergic patients with rPhl p 5b mutants

On page 50, the first full paragraph has been amended as follows:

TABLE 8:

E4

Proliferative reaction of Phl p 5b-reactive T cell clones (TCCs) from allergic patients with rPhl p 5b mutants

On page 51, the first full paragraph has been amended as follows:

✓ TABLE 9:

ES Proliferative reaction of Ph1 p 5b-reactive T cell clones (TCCs) from allergic patients

with rPh1 p 5b mutants

On page 53, the first full paragraph has been amended as follows:

TABLE 10:

E6 Proliferative reaction of the Ph1 p 5b-reactive T cell lines (TCLs) from allergic patients

with rPh1 p 5b mutants

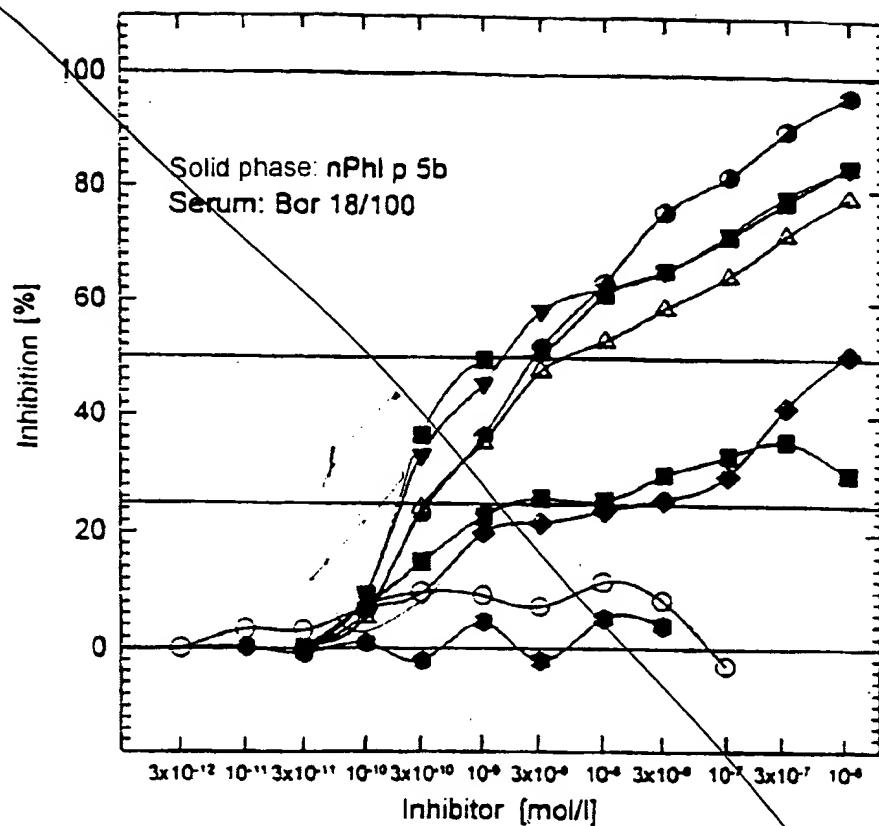


Figure 1 EAST inhibition curves of the Phl p 5b mutants using the allergic patient serum pool Bor 18/100

5 Inhibitors:

● nPhl p 5b △ PM 1 ◆ DM 1 ● DM 2
■ rPhl p 5b ▽ PM 3 ■ DM 3 ○ DM 2*

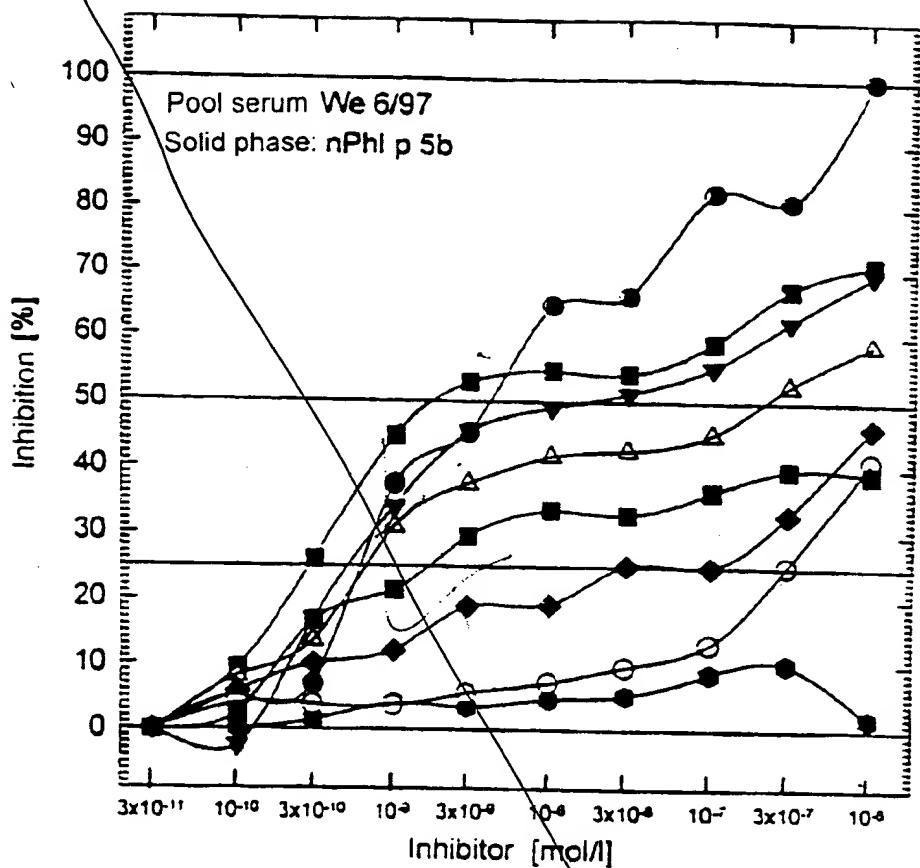


Figure 2 EAST inhibition curves of the Phl p 5b mutants using the allergic patient serum pool We 6/97

5 Inhibitors:

● nPhl p 5b ▲ PM 1 ♦ DM 1 ● DM 2
■ rPhl p 5b ▼ PM 3 ■ DM 3 ○ DM 2*

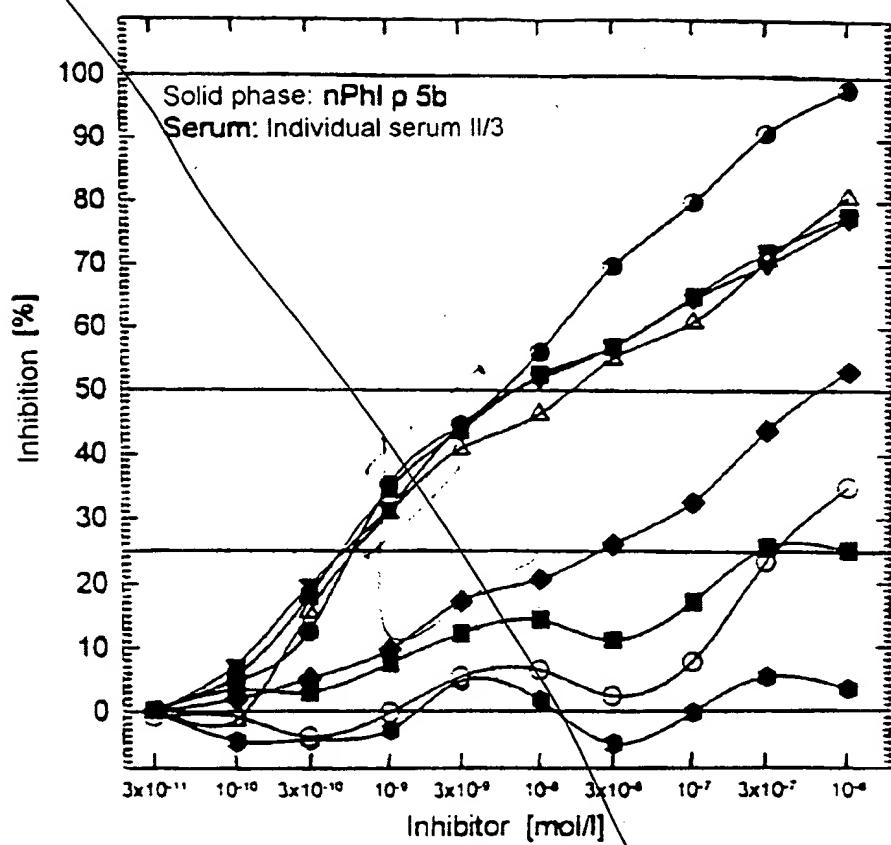


Figure 3 EAST inhibition curves of the Phl p 5b mutants using the individual allergic patient serum II/3

5 Inhibitors:

● nPhl p 5b	△ PM 1	◆ DM 1	● DM 2
■ rPhl p 5b	▼ PM 3	■ DM 3	○ DM 2-

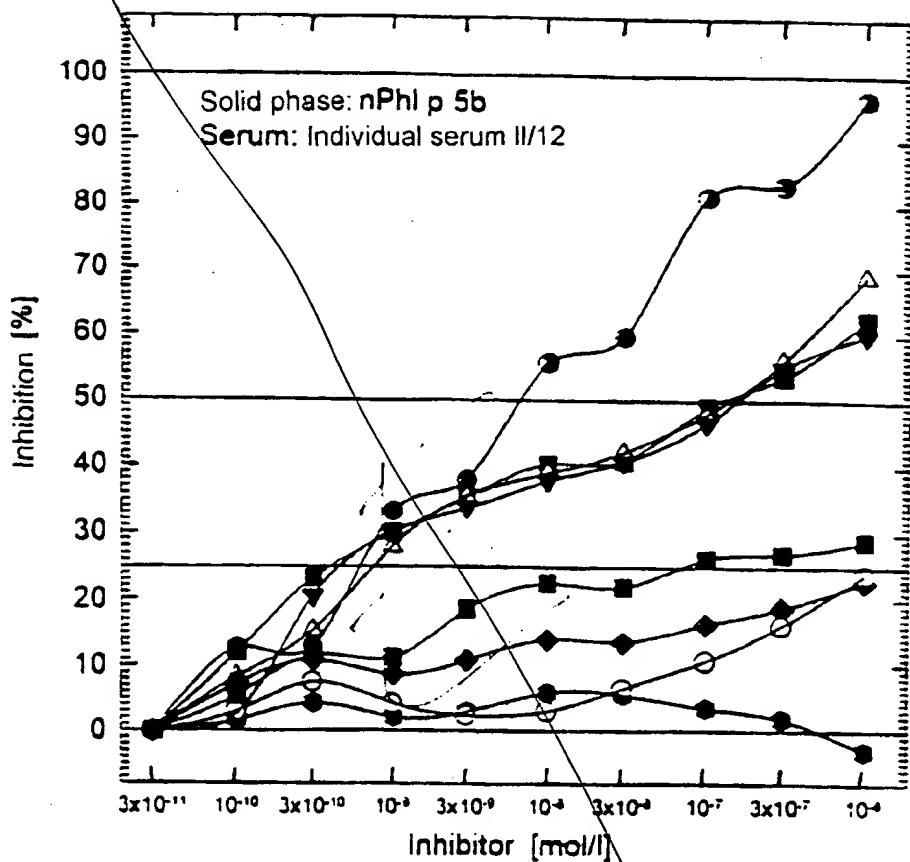


Figure 4 EAST inhibition curves of the Phi1 p 5b mutants using the individual allergic patient serum II/12

5 Inhibitors:

- nPhi1 p 5b △—△ PM 1 ♦—♦ DM 1 ●—● DM 2
- rPhi1 p 5b ▽—▽ PM 3 ■—■ DM 3 ○—○ DM 2*

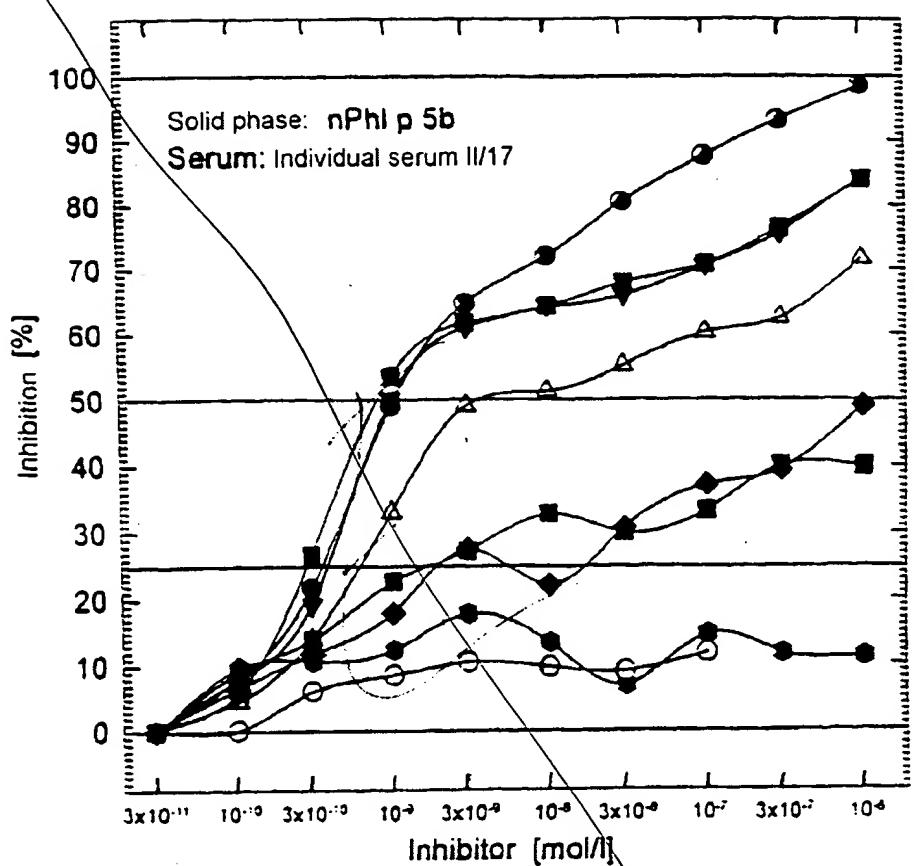
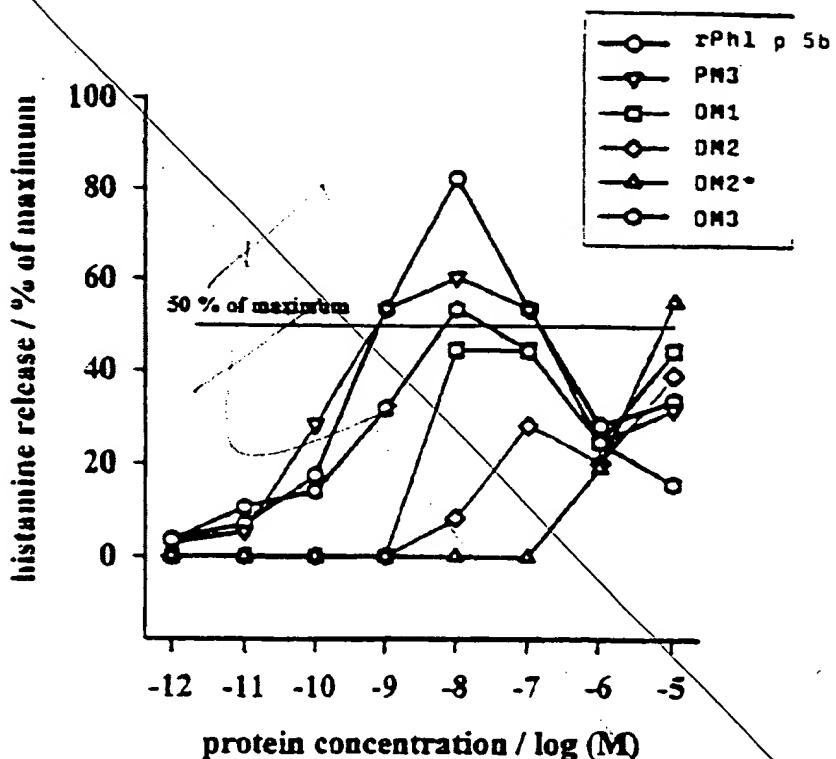


Figure 5 EAST inhibition curves of the Rhl p 5b mutants using the individual allergic patient serum II/17

5 Inhibitors:

● nPhi p 5b ▲ PM 1 ◆ DM 1
■ rPhi p 5b ▼ PM 3 ▨ DM 3 ● DM 2
 ○ DM 2*

Fig. 6 Release of histamine from human basophils after reaction with the allergens and allergen mutants



5 Example 6

Demonstration of the reactivity of recombinant Phl p 5b mutants with T cell clones from patients who are allergic to grass pollen

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The reactivity of the recombinant Phl p 5b mutants was tested on established T cell clones (TCCs) of known specificity. The TCCs derive from patients who are allergic to grass pollen (see Ex. 1) and are directed 15 against the T cell-reactive regions A (Fig. 7), B (Fig. 8) and C (Fig. 9). The T cell reactivity was measured by stimulating the clones to proliferate. The results clearly show that the TCCs react specifically with the Phl p 5b mutants when the relevant epitope is unaltered and, as expected, do not exhibit any reaction 20

when the epitope is missing or is altered by a point mutation.

Fig. 7: Proliferative reaction of Phl p 5b-reactive T cell clones (TCCs) from allergic patients with rPhl p 5b mutants

Specificity: Immunodominant T cell-reactive region A

Stimulator ¹⁾	Epitope present	Incorporation of [³ H]-thymidine into TCC ²⁾			
		JR 15	JR 13	CB 14	CB 2
Medium	-	-	-	-	-
n Phl p5	+	+++	+++	+++	+++
r Phl p5a	(±)	-	-	+	+
r Phl p5b	+	+++	+++	+++	+++
PM1	+	++	+++	+++	+++
PM3	+	+++	+++	+++	+++
DM1	+	+++	+++	+++	+++
DM2	+	+++	+++	nt ³⁾	nt
DM2	-	-	-	-	-
DM3	+	+++	+++	nt	nt
PL (12mer)	+	+++	+++	+++	+++

10 ¹ Final concentration 0.3 μM

² Stimulation index SI: < 1 (-), 1-2 (+), 2-5 (++)
5-10 (+++), > 10 (++++)

³ n.t.: not tested

Fig. 8: Proliferative reaction of Phl p 5b-reactive T cell clones (TCCs) from allergic patients with rPhl p 5b mutants

5 Specificity: Immunodominant T cell-reactive region B

Stimulator ¹⁾	Epitope present	Incorporation of [³ H] - thymidine into TCC ²⁾	
		UZH2	DW8
Medium	-	-	-
n Phl p5	+	+++	+++
r Phl p5a	(±)	±	+++
r Phl p5b	+	+++	+
PM1	+	+++	+
PM3	+	+++	±
DM1	+	+++	+
DM2	-	-	-
DM2	-	-	-
DM3	+	+++	+
PL (12mer)	+	+++	+++

¹ Final concentration 0.3 µM

² Stimulation index SI: < 1 (-), 1-2 (+), 2-5 (++)

10 5-10 (+++), > 10 (++++)

³ n.t.: not tested

Fig. 9: Proliferative reaction of Phl p 5b-reactive T cell clones (TCCs) from allergic patients with rPhl p 5b mutants

5 Specificity: Immunodominant T cell-reactive region C

Stimulator ¹⁾	Epitope present unchanged	Incorporation of [³ H]-thymidine ²⁾			SI
		1 st exp.	2 nd exp.	3 rd exp.	
Medium	-	1	1	1	-
n Phl p5	+	11.2	8.2	4.5	++
r Phl p5a	-	nt	<1	<1	-
r Phl p5b	+	11.0	7.0	5.5	++
PM1	-	<1	<1	1.1	-
PM3	+	7.4	5.9	4.5	++
DM1	+	8.6	6.2	4.4	++
DM2	+	14.4	9.1	7.1	+++
DM2	+	12.8	12.1	11.7	+++
DM3	+	9.8	6.9	4.4	++
PL (12mer)	+	20.9	15.7	nt ³⁾	+++

¹⁾ Final concentration 0.3 μ M

²⁾ Stimulation index SI: < 1 (-), 1-2 (+), 2-5 (++)

10 5-10 (+++), > 10 (++++)

³⁾ n.t.: not tested

Fig. 10: Proliferative reaction of the Phl p 5b-reactive T cell lines (TCLs) from allergic patients with rPhl p 5b mutants

TCL	Incorporation of [³ H]-thymidine into TCLs ¹							
	1 Wöl	2 Eic	3 Fre	4 Mer	5 Mah	6 Mahl	7 17.4	8 19.2
Primary stimulator	n Phl p 5	n Phl p 5	n Phl p 5	n Phl p 5	r Phl p 5a	r Phl p 5b	r Phl p 5	r Phl p 5b
Secondary activator ²								
n Phl p 5	+++	++	++	++	++	+	+++	+
r Phl p 5a	-	+	+	+	+	n.t. ³	n.t.	n.t.
r Phl p 5b	+	+	+	+	++	+	++	++
PM1	+	±	±	+	+	+	++	++
PM3	±	±	+	+	+	+	++	+
DM1	±	±	+	+	+	++	++	++
DM2	±	±	+	+	+	++	++	++
DM2*	±	±	+	+	+	++	+	+
DM3	±	±	+	+	+	+++	++	+++

35 ¹ Final concentration 0.3 μ M

² Stimulation index SI: < 1 (-), 1-2 (+), 2-5 (++) ,
5-10 (+++), > 10 (++++)

³ n.t.: not tested